REMARKS

Claims 40-70 are currently pending in the above-identified application. Claims 1-39 having been cancelled previously and Claims 51-54 and 56-70 having been withdrawn from consideration by the Examiner as drawn to a non-elected invention. Claims 40-50 and 55 are currently under consideration. The specification has been amended to correct obvious typographical and/or clerical errors. Applicants acknowledge the entry of the Withers Declaration and acceptance of the drawing filed concurrently with the application by the Draftsman. Applicants request reconsideration of the claims currently pending in the applications in light of the remarks below.

Rejections Under 35 U.S.C. §112, First Paragraph:

Claims 40-50 and 55 stand rejected under 35 U.S.C. §112, first paragraph, the Examiner believing that the claims contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors, at the time the application was filed, had possession of the claimed invention. The Examiner, believing that the only disclosed glycosynthase is the *Agrobacterium* β-glucosidase E358A mutant (AbgE358A), asserts that the issue is whether this glycosynthase "*is representative of a genus* and moreover of several highly variant genera of enzymes that do not have structural similarity thereto" (emphasis original). The Examiner further believes that the only structural limitation is "the presence of two catalytically active amino acids with carboxylic acid side chains" and that this limitation is insufficient to provide written description for "enzymes having no or insignificant similarity to AbgE358A that when mutated at said amino acids exhibit the requisite function."

Applicants initially note that, in framing the issue as whether the species exemplified in the instant application (AbgE358A) is representative of the claimed genus, the Examiner appears to have interpreted adequate disclosure of a representative number of species

as requiring an actual reduction to practice of some unknown number of examples. In this regard, and to the extent that the Examiner has not considered support for other species disclosed in the specification in determining whether a "representative number" are described, Applicants must respectfully disagree with the Examiner. Description of a representative number of species to support a genus does not require actual reduction to practice. *See* MPEP § 2163 II(A)(3)(a) ("[a]n adequate written description of the invention may be shown by *any description* of sufficient, relevant, identifying characteristics so long as a person skilled in the art would recognize that the inventor had possession of the claimed invention") (emphasis added); *see also Pfaff v. Wells Electronics, Inc.*, 48 USPQ2d 1641, 1646 (U.S. 1998) (stating that "reduction to practice is not necessary in every case").

In view of the above, Applicants respectfully disagree that the AbgE358A mutant is the only glycosynthase disclosed in the specification. First, in addition to AbgE358A, the specification also discloses two other mutant glycosidases. The first is a mutant α-amylase (human or porcine) with the aspartic acid at position 197 replaced with another amino acid such as, for example, alanine. While the second is a yeast α-glucosidase with the aspartic acid at position 216 replaced. Support for these two enzymes can be found, for example, at page 11, lines 3-7 of the specification as filed. In addition to the disclosure of other species and identifying characteristics of those species as discussed *infra*, Applicants believe that disclosure of these particular mutants must also be considered in determining whether a representative number of species has been disclosed for written description under 35 U.S.C. § 112, first paragraph.

Further, it is well settled that the written description requirement for a claimed genus is satisfied where the specification provides written description of a representative number of species by "disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus." MPEP § 2163 II(A)(3)(a)(ii). Thus, adequate written description of a genus is shown by "possession of the necessary common attributes or features of the elements possessed by the members of the genus in view of the species disclosed." *Id.* Because this standard is reviewed from the

standpoint of the skilled artisan as of the effective filing date, *see, e.g., Wang Labs v. Toshiba Corp.*, 26 USPQ2d 1767, 1774 (Fed. Cir. 1993), "an inventor is not required to describe every detail of his invention," and a disclosure "is sufficient to satisfy the requirement of section 112, first paragraph, when one skilled in the relevant art would understand what is intended and know how to carry it out." *See* MPEP § 2163 at 2100-163, *citing In re Hayes Microcomputer Products, Inc. Patent Litigation*, 25 USPQ2d 1241, 1246 (Fed. Cir. 1992).

In the present case, the relevant factors to be considered in determining whether the specification shows that Applicants were in possession of glycosynthases having the requisite function include, but are not limited to, (1) the level of skill and knowledge in the art of oligosaccaharide synthesis; (2) the level of skill and knowledge in the art relating to the partial structure, chemical properties, and functional characteristics of wild-type glycosidases; and (3) the method of making mutant glycosidases to form glycosynthases of the present invention. *See* MPEP § 2163 at 2100-163; *see also Enzo Biochem Inc. v. Gen-Probe Inc.*, 63 USPQ2d 1609, 1613 (Fed. Cir. 2002) (*en banc*). Because the invention is, for purposes of written description, "whatever is now claimed," *Vas-Cath, Inc. v. Mahurkar*, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991), Applicants initially note that independent claim 40 recites, *inter alia*, the following with respect to the glycosynthase used in the claimed method:

> ... a mutant form of glycosidase enzyme to form the oligosaccharide, said enzyme being selected from among glycosidase enzymes having two catalytically active amino acids with carboxylic acid side chains within the active site of the wild-type enzyme, and said mutant enzyme being mutated to replace one of said amino acids having a carboxylic acid side chain with a different amino acid of comparable or smaller size, said different amino acid having a non-carboxylic acid side chain ....

As used in the present application, the term "glycosidase enzyme" recited in claim 40 refers to enzymes, well-known in the art at the time of filing, having particular structural and functional characteristics including the hydrolysis of oligosaccharide products. At the time of the present invention, the amino acid sequence and crystal structures of a number of "glycosidase enzymes"

were known.[1] Also, carboxylate-containing amino acid residues recognized as within the active site of many of the "glycosidase enzymes" had been designated.[2] Still further, amino acids within the active site of many glycosidase enzymes had been identified by various other means, including the use of modified substrates that can form a relatively stable glycosyl-enzyme intermediate, *e.g.*, a 2-deoxy-2-halo glycosyl derivative (*see* page 9, lines 6-12 of the specification and Sinnott, *Chem. Rev.* 90:1171-1202 (1990) attached hereto). Although glycosidase enzymes were recognized in the art at the time of the present invention as being diverse in primary amino acid sequence and overall structure, the enzymes were known to share many structural and functional features in their active sites.[3]

Accordingly, Applicants note that, it was well known in the art that, for example many β-glucosidases and β-galactosidases had been cloned and sequenced. It was further well known that these enzymes could be grouped into several families based on amino acid sequence alignments. As indicated in the specification these sequence alignments of family members could be used to indicate the carboxylic acid-containing amino acid within the active site that are catalytically active. Applicants have provided that mutation of this carboxylic acid containing amino acid will eliminate the hydrolytic activity of the enzyme without affecting the synthase

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[1] *See generally , e.g.*, Henrissat and Bairoch, *Biochem. J.* 316:695-696 (1996) (citing pre-filing date references) (attached hereto). *See also, e.g.*, Janecek, *FEBS Lett.* 377:6-8 (1995) (describing amino acid sequence comparisons of $(\alpha/\beta)_8$-barrel glycosyl hydrolases); Jenkins *et al.*, *FEBS Lett.* 362:281-85 (1995) (comparison of crystal structures of (α/β)8-barrel glycosidases); Hengstenberg *et al.*, *FEMS Micrbiol. Rev.* 12:149-63 (1993) (reviewing 6-phospho-β-galactosidases, noting sequence similarities with other glycosidases and crystallisation of the staphylococcal and lactococcal enzymes) (each attached hereto).

[2] *See, e.g.*, Barrett *et. al.*, *Structure* 3:951-60 (1995) (pp. 953-955); Jenkins *et al.* at 282; Hengstenberg *et al.* at 159-160; Voorhorst *et al.*, *J. Bacteriol.* 177:7105-11 (1995) (pages 7107-09) (attached hereto); Janecek at 6 (Abstract and second column, noting positioning of "well-known catalytic aspartate" in $(\alpha/\beta)_8$-barrel glycosyl hydrolases); Leah *et al.*, *J. Biol. Chem.* 270:15789-15797 (1995) (page 15793, Fig. 5) (attached hereto).

[3] *See, e.g.*, Henrissat *et al.*, *Proc. Natl. Acad. Sci. USA* 92:7090-7094 (1995) (attached hereto); Jenkins *et al.*; Janecek; Mackay *et al.*, *Biosystems* 18:279-92 (1985) (noting that diversity of glucanases allows identification of "their most highly conserved (and presumably functionally important) regions" (Abstract) and showing entire conservation of certain residues by sequence alignment (Fig. 2)). *See also, e.g.*, Legler, *Adv. Carb. Chem. Biochem.* 48:319-84 (1990) (reviewing inhibition data showing diverse glycosidase enzymes to effect catalysis by the same basic mechanism) (attached hereto).

activity of the wild-type enzyme. Therefore, because a structure function relationship among glycosidase enzymes was well-known in the art, the disclosure of "glycosidase enzymes" alone reasonably conveys to the artisan a description of a genus of the mutant enzymes that can be used in the methods as claimed, irrespective of the disclosure of a particular species. *Cf.* MPEP § 2163 II(a)(3)(ii) at 2100-165.

The description provided by the specification including for example, (1) the description in the specification of the particular mutation at a catalytically active carboxylate-containing amino acid in three glycosidase enzymes, identifiable by at least four methods described in the specification, and (2) a reduction to practice of certain examples, including an actual reduction to practice, and the knowledge of the skilled artisan of the structure/function relationship between the various enzymes reasonably conveys to the artisan that Applicants were in possession of a genus of the respective mutant glycosidases as reasonably encompassed by the claims.

The specification further lists examples of glycosidases to which the methodology of the claimed invention can be employed. (*See* specification at pages 7 and 8.) Because representatives of these particular glycosidases were also known in the art as of the effective filing date, and because of the specification's disclosure regarding the mutation in glycosidases of particular carboxylate-containing amino acids within the active site to produce mutant glycosidases (the "glycosynthases"), as well as other disclosure of relevant, identifying characteristics and methods for making the enzymes as discussed *infra*, Applicants believe that the specification discloses the mutant enzymes corresponding to these other species in addition to the *Agrobacterium* β-glucosidase, human and porcine α-amylase, and yeast α-glucosidase mutants reduced to practice. Applicants believe that these species must be considered in a determination of whether a "representative number" of species are disclosed. On this basis alone, Applicants believe that the Examiner has not met the requisite burden for rejecting the claims for written description of a genus under 35 U.S.C. §112, first paragraph.

Also, the specification discloses additional, identifying characteristics of species within the claimed genus through the disclosure of partial structure, *i.e.*, the presence of two carboxylic acid groups in the active site of the enzyme (*see* specification at, *e.g.*, page 6 and

Figures 1 and 2) as well as the disclosure of mutant forms of glycosidases having these carboxylic acid groups wherein "one of the two carboxylic acid amino acids in the active site has been replaced" with a different amino acid that lacks the active carboxylic amino acid (*see* specification at, *e.g.*, pages 7 and 8). Applicants believe that this disclosure, together with known chemical properties relating to these structures and a disclosed method for determining how to achieve this structure so as to produce a glycosynthase with the requisite functional characteristics, serves to sufficiently describe the recited mutant glycosidases under 35 U.S.C. § 112, first paragraph.

In this regard, Applicants note that the nucleophilic and acid/base catalytic chemistry relating to the carboxylic acid side chains was known in the art at the time of filing. *See generally, e.g.*, Legler; Sinnott. Further, it was well-known as of the effective filing date that these carboxylic acid side chains function as nucleophiles and acid/base catalysts in the active site of glycosidases. *See, e.g.,* Legler at 364-65 and 378-79. Indeed, as of the effective filing date, methods were known in the art for identifying the nucleophile of a glycosidase using mechanism-based inhibitors that form stable intermediates with the enzyme and, in each case in which the nucleophile had been identified, the amino acid was either glutamate or aspartate.[4] Moreover, as of the effective filing date, it was well-recognized in the art that the catalytic amino acids, including the active site nucleophile, were highly conserved among glycosidases within families, and, therefore, that a preliminary identification of the catalytic carboxylate amino acids could be also be performed by sequence comparison.[5] In addition to these methods, it was known through comparison of crystal structures that the general positions of the catalytic carboxylates were conserved even across glycosidase families. *See* Jenkins *et al.*, *FEBS Lett.* 362:281-85 (1995).

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[4] *See id.* at 364-365; Wang *et al.*, *Biochemistry* 34:14554-62 (1995) (page 14554, of record). *See also, e.g.*, Miao *et al.*, *J. Biol.Chem.* 269:10975-8 (1994) (identification of active site nucleophile in human glucocerebiosidase); Trimber *et al.*, *J. Biol. Chem.* 267:10248-51 (1992) (*Agrobacterium faecalis* β-glucosidase, citing references); Tull *et al.*, *J. Biol. Chem.* 266:15621-15625 (1991) (*Cellulomonas fimi* exoglucanase) (each attached hereto).

[5] *See, e.g.*, Voorhorst *et al.* (identification of Glu372 in *Pyrococcus furiosus* β-glucosidase); Barrett *et al.* (identification of Glu397 in cyanogenic β-glucosidase from white clover); Hengstenberg *et al.* (identification of Glu375 in staphylococcal and lactococcal 6-phospho-β-galactosidase)

Further, the specification describes methods for identifying the amino acids having the catalytic carboxylates. *See, e.g.*, specification at pages 9-11. These methods include, *e.g.*, the use of modified substrates that can form a relatively stable glycosyl-enzyme intermediates; inspection of three-dimensional structure through X-ray crystallography and NMR spectroscopy; and site-directed mutagenesis coupled with determination of the glycosyl fluoride substrate utilized by the mutant enzymes. Many of these methods for identifying the catalytically active carboxylates were generally known to the skilled artisan as of the effective filing date. *See, e.g.*, Miao *et al.* (use of modified substrate to form glycosyl-enzyme intermediate); Jenkins *et al.* (use of crystal structure and sequence data to determine general position of catalytic carboxylates). In addition, in view of the disclosure in the specification as well as the art as discussed above, the skilled artisan reading the specification would recognize the described identification of the catalytic carboxylate based on the glycosyl fluoride substrate utilized by mutant enzymes (*see* specification at page 10, lines 9-23) as an additional means of determining the amino acids having the catalytic carboxylate, particularly in view of pre-filing date methods also using site-directed mutagenesis to identify catalytic amino acids in glycosidases, *see, e.g.*, Voorhorst *et al.* (indication of Glu 372 as nucleophile in *P. furiosus* β-glucosidase via site-directed mutagenesis).

In light of the above, the artisan reading the specification as filed would accept that Applicants were in possession of the recited genus of mutant glycosidases. The specification provides explicit disclosure regarding the mutation of one of the two amino acids having the carboxylic acid side chain such that the resulting mutant glycosidase is unable to hydrolize oligosaccharides while retaining "good control over the stereochemistry and regiochemistry of [the] reaction." (*See* specification at, *e.g.*, pages 7-11.) As disclosed in the specification, these mutations are designed to replace one of the carboxylic acid-containing enzymes with an amino acid lacking the carboxylic acid side chain, thereby changing the residue to an amino acid that does not act as a nucleophile or base catalyst. (*See* specification at page 8.) Thus, the disclosed mutations do not target residues unique to *Agrobacterium* β-glucosidase, nor do these mutations target molecular interactions based on, for example, alteration of enzyme conformation. Rather, the disclosed mutations are designed to alter a key functional group commonly involved in the chemistry of the hydrolysis reaction between glycosidase enzyme and

substrate. The skilled artisan reading the specification would therefore understand that these mutations, which as shown in the specification yield a mutant *Agrobacterium* β-glucosidase that can synthesize but not hydrolize oligosaccharides, would also yield mutant enzymes able to synthesize but not hydrolize oligosaccharides when applied to other glycosidases having two carboxylic acid side chains in the active site. Further, as indicated above, the site for mutation in both retaining and inverting glycosidases had already been identified or is readily identifiable using methods known in the art or disclosed in the specification. (*See, e.g.*, specification at pages 9-11.)

Therefore, the specification reasonably conveys to the artisan that Applicants were in possession of the genus of glycosynthases recited in the claims, and thus in possession of the claimed method for synthesizing oligosaccharides using the recited glycosynthases.

Accordingly, the claims satisfy the written description requirement under 35 U.S.C. § 112, first paragraph, as set forth above. In light of the above remarks, Applicants respectfully request the Examiner to reconsider and withdraw the rejection of claims 40-50 and 55 for written description under 35 U.S.C. § 112, first paragraph.

Claims 40-50 and 55 stand rejected under 35 U.S.C. § 112, first paragraph, the Examiner believing that the specification, while being enabling for the use of AbgE358A for synthesizing an oligosaccharide, does not reasonably provide enablement for the same use of any other glycosidase, including β-galactosidase. In regard to limitation of the claims to glycosidases having "two catalytically active amino acids with carboxylic acid side chains within the active site," the Examiner believes that Applicants' previous argument regarding mechanistic explanation of how the enzyme reaction occurs is not persuasive because "the explanation is related to the only example, Abg E358A," and further believes that these arguments "disregard any effect the rest of the enzyme structure has on the requisite mechanism of action as well as substrate, stereo- and regio-specificity." The Examiner asserts that the specification does not teach "how the structure of enzymes other than AbgE358A relates to function." The Examiner also states that the post-filing publications submitted by Applicants are ineffective to overcome the rejection because they were published after the effective filing date, while the enablement

must be met at the effective filing date. Similarly, the Examiner has stated the Declaration under 37 C.F.R. § 1.132 by Dr. Withers, filed June 3, 2002, is also insufficient to overcome the rejection because the declaration "describes results obtained after the filing date of the instant application."

With regard to the Examiner's statements concerning the specification's mechanistic explanation as to why the claimed amino acid change results in the change of enzyme reaction type, Applicants believe that the Examiner has not met the requisite burden under the enablement requirement. To make a rejection for enablement under 35 U.S.C. § 112, first paragraph, the Examiner has the "initial burden to establish a reasonable basis to question the enablement provided for the claimed invention." MPEP § 2164.04, *citing In re Wright*, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). Enablement is, therefore, presumed as follows:

> ... a specification disclosure which contains a teaching [of how to make and use] the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented *must* be taken in compliance with the enabling requirement of the first paragraph of § 112 *unless* there is a reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support....

In re Marzocchi, 169 USPQ 367 (CCPA 1971) (emphasis original). In this case, the disclosure describes making mutant glycosidases having glycosynthase activity by mutating one of two catalytically active amino acids having carboxylic acid side chains. The mutation disrupts the active site of the enzyme that carried out hydrolysis without impairing the catalytic activity associated with the wild-type enzyme. Examples of glycosynthases corresponding to this description and the claimed invention are exemplified by *Agrobacterium* β-glucosidase, human and porcine α-amylase, and yeast α-glucosidase mutants, *see, e.g.,* page 11, lines 1-7, and Examples 1-8). The specification further provides a teaching, based on common structural and functional features of different retaining and inverting glycosidases (involving, *e.g.*, the chemistry of carboxylic acid side chains and hydrolysis of oligosaccharides), for why these mutations achieve the described result. (*See* specification at, *e.g.*, pages 6-8.) Applicants

respectfully disagree with the Examiner's assertion that this explanation is "related to the only example, AbgE358A" because this explanation relates to the chemistry of the carboxylic acid side chains and oligosaccharide hydrolysis, which are common characteristics of the glycosidase enzymes described. In addition, since nothing more than objective enablement is required, Applicants respectfully note that the enablement of the specification is not limited by that which is exemplified: "it is irrelevant whether [the] teaching is provided through broad terminology or illustrative examples." *Wright*, 27 USPQ2d at 1513, *citing Marzocchi*, 169 USPQ at 369. Therefore, Applicants believe that the skilled artisan reading the specification would interpret the specification as providing a teaching for how to make and use glycosynthases that reasonably corresponds in scope to those described in the specification and recited in the claims. Thus, the specification is presumed enabled absent a reasonable basis for doubting the objective truth of the statements therein.

Applicants believe that the Examiner has not sufficiently rebutted this presumption. The Examiner believes that Applicants' arguments with respect to the mechanistic explanation are not persuasive because "[t]hese arguments disregard any effect the rest of the enzyme structure has on the requisite mechanism of action as well as substrate, stereo- and regio-specificity." However, the Examiner has merely made a general assertion regarding protein structure and specificity without addressing the particular teachings within the specification, including, for example, the nucleophilic and acid/base catalyst activities involved in the glycolytic chemistry pertinent to the claimed invention. The Examiner has provided no evidence or reasoning why the "rest of the enzyme structure" in different glycosidases would affect the results described in the specification regarding glycosynthase activity.

In addition, Applicants respectfully note that the post-filing references and declaration submitted with the previous response of May 21, 2002, demonstrate that different mutant glycosidases as described in the specification yield the requisite glycosynthase activity, regardless of the "rest of the enzyme structure." As discussed below, Applicants believe that these references and the declaration under 37 C.F.R. § 1.132 by Dr. Withers, filed June 3, 2002 (herein "Withers Declaration"), are properly admissible and are sufficient to demonstrate enablement of the specification under 35 U.S.C. § 112, first paragraph.

First, Applicants strongly disagree with the Examiner's position regarding the sufficiency of the Withers Declaration. This declaration was not offered to supplement the specification as filed but, rather, as evidence of the level of skill in the art at the time of filing and that the disclosed mutated glycosidase would have been operative as described. As such, the Declaration is admissible as evidence that the specification as filed is enabling for the claimed invention. *Cf., e.g., Gould v. Quigg*, 3 USPQ2d 1302, 1305 (Fed. Cir. 1987) (holding post-filing publication is admissible where "offered as evidence of the level of ordinary skill in the art at the time of the application and as evidence that the disclosed device would have been operative"). The Declaration provides evidence that the claimed invention was operable as of the filing date using techniques known in the art at the time of filing and the methods disclosed in the specification. In particular, Applicants respectfully note that, in the experiments to which Dr. Withers testified in the Declaration, the catalytically active glutamic acid residue at amino acid 537 of *E. coli* Lac Z β-galactosidase was replaced with serine, a smaller amino acid lacking a carboxylic acid side chain. The activity of the glutamic acid residue at this position was known in the art prior to the effective filing date of the present application (*see* Henrissat *et al.*, *supra*). This mutation is precisely that disclosed in the specification for achieving a glycosynthase as recited in independent claim 40. (*See, e.g.*, specification at page 8, where the specification describes the mutation of one of two catalytically active, carboxylic acid side chain-containing amino acids to an amino acid not having the carboxylic acid, particularly amino acids of smaller or equal size.) In addition, the glycosynthase resulting from disabling the active site for hydrolysis is demonstrated to be capable of coupling an α-glycosyl donor molecule (α-D-galactosyl fluoride) with a glycoside acceptor molecule (β-D-galactopyranoside or β-D-cellobioside) (*see* Withers Declaration, ¶¶1, 5, and 6), as disclosed in the specification (*see* specification at, *e.g.*, pages 12 and 13).

Accordingly, because the testimony in the Withers Declaration relates to methods described in the specification as filed and further demonstrates that β-galactosidase, when mutated according to the disclosure in the specification, would be operable as a glycosynthase, Applicants believe that this declaration is properly admissible as evidence demonstrating enablement of the claimed methods as of the effective filing date.

Similarly, Applicants respectfully disagree with the Examiner's position regarding the admissibility of the post-filing publications. As with the Withers Declaration, these publications were not submitted to supplement the disclosure in the specification with a later-existing state of art but, instead, as evidence that the invention as claimed was operable as of the filing date using the methods disclosed in the specification. Post-filing publications are admissible as evidence of the state of the art existing on the filing date of the application, *e.g.*, *In re Hogan and Banks*, 194 USPQ 527, 537 (CCPA. 1977), and as evidence that the disclosed invention would have been operative as of the effective filing date, *e.g.*, *Gould*, 3 USPQ2d at 1305. *See also In re Pottier*, 153 USPQ 407, 408 n.1 (CCPA 1967).

Therefore, Applicants again respectfully refer the Examiner to the following, previously submitted references described in the Declaration of Dr. Withers filed with this response: Nashiru *et al.*, *Angew Chem. Int. Ed.* 40:417-420 (2001); Trincone *et al.*, *Bioorganic & Medicinal Chem. Lett.* 10:365-368 (2000); Mayer *et al.*, *FEBS Lett.* 466:40-44 (2000); Mayer *et al.*, *Chem. & Biol.* 8:437-443 (2001); Fort *et al.*, *J. Amer. Chem. Soc.* 122:5429-5437 (2000); and Malet *et al.*, *FEBS Lett.* 440:208-212 (1998). Each of these references describes a mutant glycosidase, different than the AbgE358A mutant, in which an active site carboxylic acid-containing amino acid is converted to an amino acid which does not contain a carboxylic acid side chain. The mutant glycosidases reported by these references are briefly summarized below:

(1) Nashiru *et al.* disclose a β-glycosynthase derived from a mannosidase cloned from *Cellulomonas fimi* (one of the types of glycosidases listed on page 7 of the application), in which the active site nucleophile Glu519 was converted to either alanine or serine. Both provided an enzyme that was inactive as a hydrolase but retained mannosynthase activity. The active site nucleophile was identified by trapping the covalent mannosyl-enzyme intermediate with 2-deoxy-2-fluoro β-D-mannosyl fluoride, followed by proteolysis and sequencing of the labeled peptide (a method described in the specification).

(2) Trincone *et al.* disclose a glycosynthase, derived from the β-glycosidase of *Sulfolobus solfataricus*, in which that active site

glutamic acid residue (Glu387) of the wild-type enzyme is mutated to either alanine or glycine. The nucleophile had been previously identified as Glu387 using various methods, such as use of a conduritol B epoxide inactivator (a method known in the art as of the effective filing date[6]).

(3) Mayer *et al.* (2000), describes a Glu358Ser mutant of *Agrobacterium* β-glycosidase (a different mutant of the enzyme in the specific examples of the application replacing the carboxylic acid-containing amino acid (Glu) with a non-carboxylic acid-containing amino acid that has a side chain of approximately equal or smaller size) which has improved glycosynthase activity resulting in higher yields, reduced reaction times, and enhanced synthetic repertoire.

(4) Mayer *et al.* (2001) describes mutants Glu358Cys and Glu358Gly of *Agrobacterium* β-glycosidase (also different mutants of the enzyme in the specific examples of the application replacing the glutamic acid with additional amino acids having a side chain of approximately equal or smaller size) and shows them to have no hydrolysis activity and different rates of glycosynthase activity.

(5) Fort *et al.* describes a glycosynthase prepared by replacing the catalytic nucleophile Glu197 in endonuclease Cel7B from *Humicola insolens* with alanine. Glu 197 had been identified as the catalytic nucleophile by trapping of the covalent glycosyl-enzyme intermediate and comparison of amino acid sequence with other related glycosidase family members (methods described in the specification).

(6) Malet *et al.* describes mutant forms of glucanases from *Bacillus licheniformis*. The paper notes that two glutamic acid residues Glu138 and Glu134 had been identified as the catalytic acid/base and the nucleophile, respectively, citing a pre-filing date reference (1994).

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[6] *See, e.g.*, Legler *et al.* at 364-65.

The mutant form Glu134Ala was prepared and shown to lack hydrolysis activity and to act as a glycosynthase.

The mutations described above and the resulting inactivation of hydrolysis activity and maintenance of glycosynthase activity are as described in the specification as filed. (*See, e.g.*, specification at page 8, where the specification describes the mutation of one of two catalytically active, carboxylate-containing amino acids to an amino acid not having the carboxylic acid.) The sites for mutation of the wild-type enzyme either had been identified as of the effective filing date or were determined using methods described in the specification or known in the art. Therefore, with respect to making a glycosynthase by modifying one of two catalytically active amino acids having a carboxylate, these references do not add to the specification and knowledge of the art beyond that of the effective filing date. Applicants believe it is also apparent from these disclosures that the time and effort to produce the glycosynthases described in the references was not more than would be expected using the knowledge and skill in the art at the time of filing and the methods described in the specification. Furthermore, these references clearly show that the modification to a glycosidase at one of two catalytically active amino acids having carboxylic acid side chains as described in the specification as filed result in an enzyme which can be used in the method of claim 40. For these reasons, Applicants believe that the post-filing references are admissible as additional evidence of enablement. In light of the remarks above, Applicants respectfully request the Examiner to reconsider the present rejection as claims 40-50 and 55 are fully enabled by the specification as required under 35 U.S.C. § 112, first paragraph.

Double Patenting Rejection

Claims 40-50 and 55 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatenable over claims 1-17 of U.S. Patent 5,716,812 and over claims 1 and 2 of U.S. Patent 6,284,494. Applicants respectfully note the Examiner's rejection. As stated in the response of May 21, 2002, to the previous Office Action, Applicants will submit an appropriate terminal disclaimer, should it be determined that it is required, upon receiving an indication that the claims are otherwise in condition for allowance.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 206-467-9600.

Respectfully submitted,

Dated: 21 February 2003

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